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34. The process according to claim 27, characterized in that the separation medium is continuously regenerated and equilibrated, simultaneously with the separation of the plasma proteins.

B1 cont.

35. The process according to claim 27, characterized in that, when a material for adsorption chromatography is used as the separation medium, at least two different eluents are simultaneously passed through said separation medium having the annular design.

36. The process according to claim 27, characterized in that at least two different separation media are employed in layers.

37. The process according to claim 27, characterized in that a polymeric block material is employed as said separation medium.

REMARKS

Claims 27-37, presented hereby in place of claims 14-26, are pending.

Claim 27 combines subject matter from claims 14, 15, and 17, revised to more clearly define the subject invention, as explained below. Claims 30 and 31 contain subject matter from claim 20, revised as explained below. Claims 28, 29 and 32-37 correspond to claims 18, 19, and 21-26, respectively, made dependent on claim 27.

Claim 27 adds to the subject matter of claim 14 the embodiments of claims 15 and 17 and, besides, the "spherical particles" of the "application medium" are further characterized by the feature "with a hydrophobic surface," which is disclosed in the specification on page 4, 3rd paragraph.

As represented by claim 27, claim 14 is limited to separation and/or isolation of "human" plasma proteins, the source of which is limited to "blood plasma" or "a mixture containing virus inactivated plasma proteins." Furthermore, the "layer of application medium," on top of the separation medium, comprises "spherical particles with a hydrophobic surface."

Applicants submit that due to the limitations added to claim 14 in accordance with claim 27, the references cited in the rejections of record under §102(b) are patentably distinguished.

First, the rejections under 35 USC 102(b) based on U.S. Patent No. 5,639,376 (Lee) and *Journal of Chemical Engineering of Japan, 25*, 403-407 (1992) (Takahashi) are overcome by incorporating claim 15 into claim 14, i.e., as claim 27. Claim 15 was not rejected based on Lee or Takahashi.

Secondly, reconsideration is requested with respect to the rejection of claim 15 under 35 USC 102(b) based on *Ind. Eng. Chem. Res., 30*, 1061-1067 (1991) (Bloomingburg) for the following reasons.

The presently claimed invention is novel over Bloomingburg, because Bloomingburg is directed to the separation of two purified model proteins, which are mostly albumin and hemoglobin. Bloomingburg is not directed to the purification of human plasma proteins from blood plasma or from a mixture containing virus inactivated plasma proteins. The observation in the statement of

rejection that Bloomingburg is directed to a purification from blood plasma, citing page 1061, col. 2, line 14, is not correct. In this passage, only albumin, hemoglobin and cytochrom C are cited, which are not "blood plasma". Blood plasma is a complex mixture of numerous proteins and other components.

According to Bloomingburg, the proteins are separated by passing through a resin. On top of the resin, as described in the passage cited in the statement of rejection (page 1062, col. 1, lines 8-14), an application medium is packed. The application medium is "Dowex Monosphere", which is "a gel-type cation-exchange resin with a diameter of approx. 300 μm". Contrary to the allegation in the statement of rejection, the application medium does not comprise "spherical particles". The passage cited in the statement of rejection (page 1062, col. 2, line 11), referring to "spherical particles," concerns the separation medium, which is S-Sepharose (see page 1062, col. 1, first sentence in paragraph "Materials": "S-Sepharose (Pharmacia) resin consists of..."). In contrast, as indicated, the application medium is of "a gel-type". Spherical particles, on the other hand, are solid and thus different and distinct from a "gel-type" resin. Furthermore, the spherical particles in the presently claimed invention have a "hydrophobic surface." Even if the application medium of Bloomingburg comprised spherical particles, the application medium is a hydrophilic cation-exchange resin.

The aim of the present inventors was not to separate purified model proteins of high stability. On the contrary, the aim of the inventors was to separate labile, human-blood-plasma proteins from crude protein mixtures, which are blood plasma and virus-inactivated mixtures. The inventors had

faced the problem that, when applying techniques of the state of the art for the separation of such mixtures, when using glass beads as the application medium, the activity of various important protein components of such mixtures is altered significantly. The inventors faced the problem that the process of Takahashi et al., though possibly applicable for the separation of relatively stable purified model protein such as myoglobin and hemoglobin, is not applicable for the preparation of human plasma proteins from blood plasma or virus-inactivated protein mixtures.

The inventors of the presently claimed invention realized that, surprisingly, for the purification of human plasma proteins, even from crude mixtures comprising various proteins, the use of an application medium of spherical particles with a hydrophobic surface is applicable and advantageous. The activity of the protein components remain basically unaltered, in contrast to what was observed by methods of the prior art. The state of the art clearly taught away from such a solution. The inventors actually did the opposite of what was taught, i.e., by using hydrophobic instead of hydrophilic surfaces.

In order to substantiate these findings, the inventors performed comparative examples, which are submitted herewith as Appendix. The comparative examples show how the activity of various important proteins from plasma pools is altered when they are incubated with glass beads according to the prior art (series C), and how the activity remains, essentially, unaltered, when they are incubated with hydrophobized glass beads, which are silicon oil treated (series B). A comparison with series A, wherein no beads are applied as a control, shows that the application medium of the

invention has almost no effect on the activity of the plasma pool proteins. Therefore, they can be used as an application medium to prevent overflow and mixing of the eluents.

Favorable action is requested.

Respectfully submitted,

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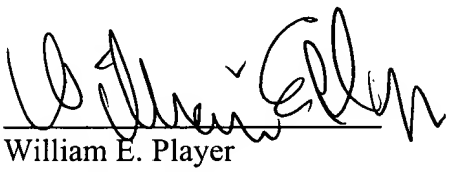
By: 

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Experimental procedure to compare suitability of silicon-treated glass beads versus non treated beads to be used for continuous separation of human plasma

Experiment: 2GG1505

Experimental set up:

Approximately 3 ml plasma samples (in-process sample: plasma pool; final product) were treated as follows:

A: no treatment

B: incubation with silicon-oil treated glass beads

C: incubation with untreated glass beads.

Samples:

Plasma pool: batch # 118087950, # 117086950, Poolmix # 116001930

Plasma (final product): batch # 116001930, # 116002930, # 116003930

glass beads:	silicon oil treated,	ca. 0.5g/vial,	Experiment B
glass beads:	untreated,	ca. 0.5g/vial,	Experiment C

Temperature: ambient temperature

Incubation time: approx. 6 hrs. under constant agitation

After incubation glass beads were sedimented by centrifugation at 2000 rpm (acc. to ca. 900 x g) for ca. 30 sec.

Sample aliquots were analyzed immediately.

Test parameter:

FVIII: C clotting and chromogenic activity, FV, FVII, FVIIa, FX

Appendix

Silicon-oil treatment:

Approx. 20 g of glass beads (150-250 µm, provided by PRIOR®) were washed with 0.5 N NaOH, 5 min. and rinsed with WFL until pH <8 was reached.
Glass beads were transferred into 100 ml WFL containing 1% silicon oil (Baysilon) and heated for 20 min. at 100 °C under stirring. Subsequently, sedimented glass beads were washed several times with cold WFL and dried at 100 °C in a heated chamber for 1 hour. Finally, glass beads were further dried under vacuum at < 0.1 mbar for 15 min.
Beads without treatment were prepared as above omitting silicon oil treatment.

Incubation of samples with glass beads:

Approx. 0.5 g of glass beads was used to be incubated with ca. 3 ml of samples (in PP tubes, ambient temperature) under gentle rotation.
Control samples were treated identically but without addition of glass beads.

Result:

Table 1 gives results of biological parameters measured in Plasma pool (i.e. Starting material).

Series -A represent control samples series -B silicon oil treated beads, series -C untreated beads

Table 1:

Plasmapool starting material	*FVIII:C o. st. U/ml*	*FVIII:C chr. U/ml*	*FVII U/ml*	*FVIIa mU/ml*	*FV U/ml*	*FX U/ml*
118087950/A	0.6	0.4	0.9	102	0.8	0.8
118087950/B	0.5	0.5	1.28	270	0.8	0.8
118087950/C	0.5	0.4	**4.9**	**3018**	**1.3**	**1.2**
117086950/A	0.5	0.5	0.7	67	0.7	0.8
117086950/B	0.5	0.6	0.9	140	0.7	0.8
117086950/C	0.5	0.5	**4.9**	**2274**	**1.2**	**1.1**
116001930/A	0.7	0.5	1	128	1	1
116001930/B	0.6	0.5	1	205	1	1
116001930/C	0.5	0.6	**1.6**	**368**	**1.1**	**1**

Appendix

Table 2 gives results of biological parameters measured in Plasma (i.e. Final product).

Series -A represent control samples series -B silicon oil treated beads, series -C untreated beads.

Table 2:

Plasma	*FVIII:C o. st.*	*FVIII:C chr.*	*FVII*	*FVIIa*	*FV*	*FX*
Final product	U/ml	U/ml	U/ml	mU/ml	U/ml	U/ml
116001930/A	0.6	0.5	0.9	90	0.9	1
116001930/B	0.6	0.5	1	100	0.9	1
116001930/C	0.5	0.5	**2.5**	**998**	**1.3**	**1.2**
116002930/A	0.6	0.6	1	90	0.9	1
116002930/B	0.6	0.6	1.1	140	1	1
116002930/C	0.6	0.4	**2.8**	**1059**	**1.3**	**1.4**
116003930/A	0.6	0.5	1	83	1	1
116003930/B	0.6	0.6	1.1	143	1	1
116003930/C	0.5	0.5	**2.3**	**761**	**1.3**	**1.3**

In both the starting material and the final product there is a tremendous impact on the biological activity of FVII and FVIIa upon incubation with untreated glass beads. A 3- to 30-fold increase of FVIIa could be measured. In the case of FVIIa 2- to 6-fold increase could be observed. FV and FX are effected as well, however, the increase of these particular parameters were less pronounced. A minute change in parameters could also be observed with silicon oil treated beads, however, compared to untreated control samples these changes can rather be neglected. FVIII:C activity was obviously not influenced.

Appendix

Conclusion:

Glass beads were suggested to be used to cover the gel bed in annular chromatography. This seems to be a suitable method to avoid destruction of the column bed and allow application of sample.

The results given in tables 1 and 2 clearly demonstrate that the contact of plasma proteins with untreated glass beads (e.g. during the separation by annular chromatography) leads to changes (i.e. most probably activation) of biological properties.

Silicon oil treated glass beads, however, do not show remarkable changes when used for this particular experimental set up. Consequently, the results strongly indicate the necessity to treat the glass beads before use in order to avoid changes/activation of plasma derived proteins.

Appendix